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Electric Organ Discharge and Electrosensory Reafference in Skates

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Abstract. Skates possess bilateral electric organs that produce intermittent, weak discharges of relatively long duration compared to the discharges of other weakly electric fish. They, like all elasmobranchs, also have an electrosensory system capable of detecting weak, low-frequency electric fields. Several studies have suggested that the discharge is used in some type of social communication. This study measured the strength and nature of the response of the skate electrosensory system to electric organ discharge.

Electric organ discharge (EOD) was elicited *via* electrical stimulation of the medullary command nucleus in two species of skates. The temporal structure and power spectra of the EODs demonstrated that they should be effective stimuli for the skate electrosensory system. The responses of electrosensory afferent fibers in the anterior lateral line nerve (ALLN) to EODs were variable depending upon the location and orientation of the receptor. The responses of most ALLN fibers were very weak compared to the strong reafference produced by the skate's ventilatory activity. Unlike the common-mode ventilatory reafference, EOD reafference was variable in terms of excitation or inhibition, depending upon receptor orientation. Despite the low signal-to-noise ratio observed in ALLN responses to EODs, it is likely that EODs serve as a communicative signal over moderate distances.

Introduction

Marine elasmobranchs of the skate family (Rajidae) possess electric organs that generate a weak electric organ discharge (EOD) (for reviews of electric organs see Grundfest and Bennett, 1961; Bennett, 1971; Bass, 1986). Although the existence of these organs has been known since the last century (Stark, 1844; Ewart 1888a, b, 1892), the behavioral function of these organs has yet to be conclusively determined. Owing to its small amplitude (Bennett, 1971; Bratton and Ayres, 1987), the EOD of the skate is unlikely to be used in prey capture, unlike the discharge of the larger and more powerful electric organ found in rays of the genus *Torpedo*. Furthermore, unlike the nonhomologous electric organs of freshwater teleost fishes (notably the gymnotids and mormyrids), the skate electric organ is only intermittently active and is more variable in amplitude and temporal discharge pattern (Bennett, 1971; Bratton and Ayres, 1987). Although an intra- or interspecific communicative function for this discharge has been suggested (Mikhailenko, 1971; Mortenson and Whitaker, 1973; Bratton and Ayres, 1987), the precise role of such communication in the skate's ethogram remains to be conclusively determined.

The electric organs of the skate are spindle-shaped structures that extend bilaterally along the length of the longitudinal axis of the tail (Stark, 1844; Ewart, 1888a; Sanderson and Gotch, 1888) (Fig. 1). The organ consists of approximately 8,000–10,000 cup- or disk-shaped electrocytes arranged anteroposteriorly in series (Ewart, 1892; Brock *et al.*, 1953; D. M. Koester, Univ. of New England, pers. comm.). Each electrocyte develops a weak potential when depolarized by the innervating nerve fibers (Bennett, 1971). Previous studies have demonstrated that the electric organ discharge of various skate species is of variable amplitude and duration, but generally consists of a monophasic, head-negative wave of 60–500 ms duration (Bennett, 1971; Bratton and Ayres, 1987). The electrocytes themselves are not electrically excitable overall, although the posterior face of the cup-shaped electrocytes possesses an electrically excitable component that involves a delayed rectification (Bennett, 1971). Electrocytes in the skate electric organ are innervated by motor neurons in the

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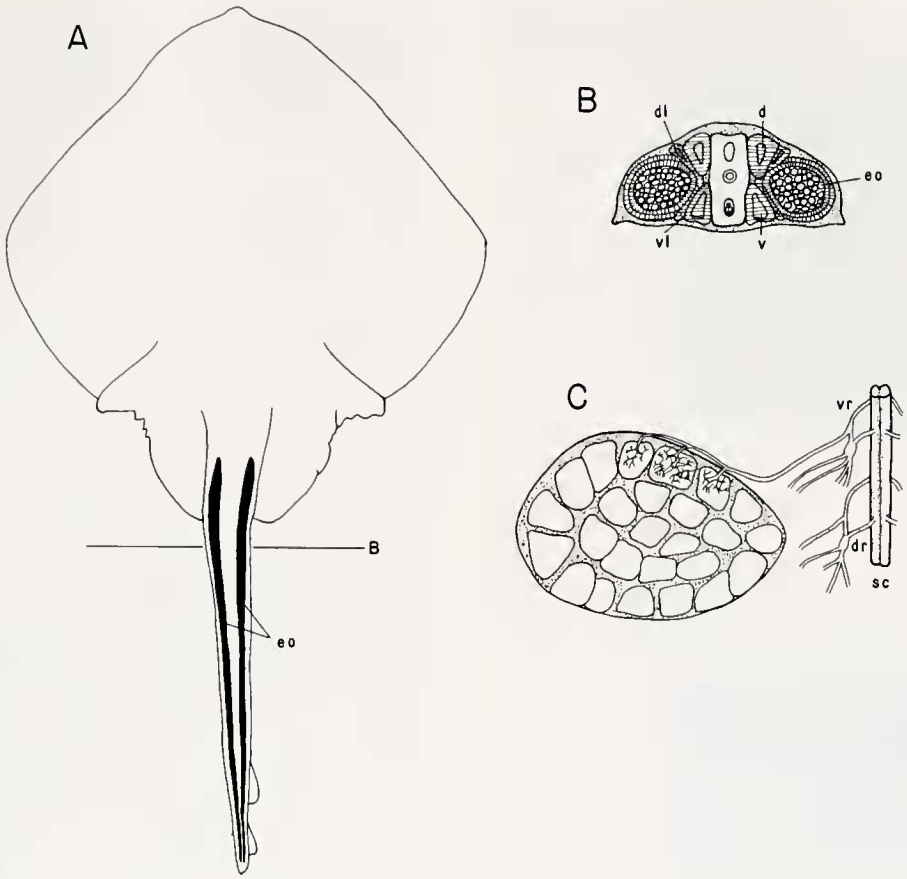

Figure 1. Location and innervation of the electric organs in *Raja*. (A) Location of electric organs (in black) in the tail of *Raja*. (B) Transverse section through the tail of *Raja* at the level indicated in A, showing the electric organs, muscles, and vertebral column. (C) Innervation of electrocytes by spinal electromotor nerves. (Modified from Ewart, 1892; and Bratton and Ayers, 1987.) Abbreviations: d, dorsal muscle group of epaxial musculature; dl, dorsolateral muscle group of epaxial musculature; dr, dorsal root of spinal nerve; eo, electric organ; sc, spinal cord; v, ventral muscle group of hypaxial musculature; vl, ventrolateral muscle group of hypaxial musculature; vr, ventral root of spinal nerve.

ventral spinal cord (Koester, 1987; Baron *et al.*, 1992), which in turn are believed to receive direct descending input from an electric organ command nucleus (EOCN) located in the basal midline of the medulla (Albe-Fessard and Szabo, 1955; Szabo, 1955, 1961). The location and nature of any higher level descending projections to the EOCN are unknown, although reflexive EODs in response to tactile stimulation can still be elicited in skates following ablation of the telencephalon or the cerebellum (Albe-Fessard and Szabo, 1955).

Skates, like other elasmobranchs, also possess an electrosensory system capable of detecting the very weak electric fields produced by physicochemical and biological phenomena in aquatic environments (for reviews, see Bodznick and Boord, 1986; Kalmijn, 1988). The electrosensory receptor organs, the ampullae of Lorenzini, are innervated by fibers of the anterior lateral line nerve (ALLN), which project to and terminate within the first-order electrosensory nucleus of the medulla, the dorsal octavolateralis nucleus (DON). The ampullae themselves consist of a long tubelike canal that terminates internally in a bulbous alveolus and externally through a pore in the epidermal surface (for reviews of ampullary electroreceptor structure and function, see Murray, 1974; Bennett and Clusin, 1978; Sejnowski and Yodlowski, 1982; Bodznick and Boord, 1986). The apical membranes of the receptor cells themselves are embedded in the base of the alveolus and connected to the walls of the alveolus by tight junctions. The walls of the alveolus and canals have a high electrical resistance, and the internal lumen of the ampulla is filled with a highly conductive, K^+-rich, jelly-like matrix (Murray and Potts, 1961). The ampulla thus functions as an insulated core conductor, and the receptor cells measure the voltage drop across the length of the ampulla; *i.e.*, the receptors measure the potential difference between the internal lumen, which is isopotential

with the external environment at the epidermal pore, and the internal field gradient established within the animal by the low skin resistance and high conductivity of the internal tissues.

The electrosensory system of elasmobranchs is extremely sensitive; the behavioral threshold for responses to dipole sources is less than 5 nV/cm (Murray, 1974; Kalmijn, 1982). The electrosensory system is most sensitive to low-frequency electric fields, from near direct current (DC) (<0.01 Hz) to approximately 10–15 Hz (Montgomery, 1984a; New, 1990; Tricas and New, unpub. obs.). Behavioral studies have demonstrated that the electrosensory system is used in directing prey-catching behavior; elasmobranchs will strike at electrical sources that mimic the bioelectric fields produced by living organisms in water (Kalmijn, 1971, 1982). Other studies suggest that elasmobranchs may also use their electrosense to detect electric fields induced by movement relative to the earth's magnetic field (Kalmijn, 1978). Additionally, recent experiments in a mating population of stingrays (which lack an electric organ) demonstrated that males search for and locate females hidden beneath a sand substrate by detecting the weak ventilatory potentials produced by the females (T. Tricas, Florida Inst. Tech., pers. comm.). The ability of male stingrays to detect the very weak signals of females (approximately 5 μV intensity at 1 cm from the spiracle) at a distance (about 1 m) suggests that the electric organ in skates may serve a useful purpose in social communication (Mikhailenko, 1971).

The purpose of this study is to characterize the EOD and examine the responses of the skate electrosensory system to discharge of the animal's own electric organ, with specific regard to the usefulness of this organ in signaling other skates and to the nature and amount of reafference it produces. Previous studies have indicated that the ventilatory potentials produced by elasmobranchs are a significant source of electrosensory reafference (Montgomery, 1984a; New and Bodznick, 1990; Bodznick and Montgomery, 1992; Bodznick *et al.*, 1992). This internally generated reafference modulates the activity of electroreceptors by changing the voltage at the internal face of the apical membrane of the receptor cells. These internal potentials thus provide a source of common-phase reafference; all receptors are excited or inhibited in phase with one another regardless of receptor orientation or position on the body surface. Whether the electric organ discharge of the skate also constitutes a significant source of internal reafference, *via* a similar low-resistance internal pathway, is also investigated in this study.

Materials and Methods

Specimens of little skate, *Raja erinacea* (n = 18), and winter skate, *Raja ocellata* (n = 2), were collected by otter trawl in the vicinity of Woods Hole and the Elizabeth Islands, Massachusetts. The specific identity of individual skates was determined by counting the teeth series in each jaw (Bigelow and Schroeder, 1953). The animals were held in temperature-controlled (16°C) running seawater tanks prior to experimentation.

Individual skates were anesthetized in approximately 0.025% tricaine methanesulfonate (MS-222) and the dorsal aspect of the brain and anterior lateral line nerve (ALLN) exposed. In some cases, the thoracic spinal cord, the rostral and caudal poles of the electric organ, or all three, were exposed at one or two locations for implantation of stimulating electrodes. The animal was decerebrated by a complete transection of the diencephalon and caudal telencephalon at the level of the optic chiasm. The animal was then placed in an acrylic holder designed to hold the animal immobilized during the experiment. Immobilizing drugs such as curare were not employed in this study because they block synaptic transmission at the spinal motor nerve–electric organ electrocyte junction. The holder was positioned in an acrylic tank (61 cm × 12.5 cm × 45.5 cm), and cooled (12°C), recirculated, aerated seawater was passed over the gills by means of an oral tube to ensure an adequate supply of oxygen. A small pulse of fast green dye was injected into the oral tube to ensure that water was passing over the gills and not back out of the mouth or spiracle.

The discharges of the electric organ were measured through Ag-AgCl wire electrodes bilaterally or unilaterally implanted subcutaneously at about the level of the rostral and caudal poles of the electric organ in the tail. The discharges were amplified and recorded on tape with an instrumentation recorder (Vetter, Model B) for subsequent analysis. The spread and orientation of the internal and external fields generated by discharge of the electric organ were measured directly by electrodes either in the seawater bath or implanted in the ampullary clusters within the body of the skate (see below). External fields were measured using an Ag-AgCl wire electrode positioned at various locations around the animal's body in the seawater bath. A reference electrode was positioned at some distance (approximately 25 cm) from the animal. Both electrodes were connected to a Grass P-15 differential preamplifier, filtered between 0.3 Hz and 1.0 kHz (time constant >150 ms), and measured directly on an oscilloscope. Internal potentials were measured by implanting an insulated Ag-AgCl tipped wire electrode into the subdermal clusters of ampullary alveoli in which the electroreceptors are located. The recording electrodes were introduced through a fine hypodermic needle inserted through the skin at a point on the animal above the water line in the tank and then withdrawn. The reference electrode was then positioned at various locations around the skate's body to measure the voltage drop across the length

of the receptors (in a manner similar to the voltage measured by the receptors). The potential difference between recording (internal) and reference (external) electrodes was amplified by a Grass P-15 preamplifier and measured on an oscilloscope.

The activity of individual ALLN fibers was recorded by means of glass micropipette electrodes filled with 2 *M* NaCl saturated with fast green dye and with input impedances of 12–20 megohms. Responses of ALLN fibers were recorded directly from the nerve, near the ALLN ganglion. Electrosensory ALLN fibers were initially identified by their responses to a uniform electric field oriented along the longitudinal axis of the tank between two carbon rod electrodes connected to a Grass Instruments S-88 stimulator. The field stimulus consisted of DC-step pulses of approximately 100 μV/cm amplitude and 700 ms duration. The intensity of the field was measured directly in the tank by means of two Ag-AgCl wire electrodes positioned 10 cm apart and parallel with the orientation of the field. The threshold receptive fields of the individual ALLN fibers were subsequently identified with a hand-positioned local dipole source consisting of two 2.0-mm (o.d.) glass tubes filled with 2% agar in seawater and connected to a Grass Instruments S-88 stimulator *via* Ag-AgCl wire electrodes. The initial intensity of the local DC-step field, measured directly at one of the poles, was <50 μV, and the average distance between the poles of the local dipole was 4.0 cm. Upon encountering ampullary pores responding to local electric field stimuli, the stimulus was systematically decreased to identify as nearly as possible the threshold receptive field of the recorded ALLN fiber. In this study, the threshold receptive field is defined as the location of the epidermal pore of the ampullary electroreceptor, but in actuality the "receptive field" of the electroreceptor is the distance between the pore and the electroreceptor cells within the internal alveoli across which the voltage drop is measured. Action potentials recorded from ALLN fibers were transformed into digital logic pulses through a WPI window discriminator and stored as peristimulus time histograms on a Tracor Northern signal analyzer. Additionally, spike activity and electric organ discharges were in some cases recorded as analog signals on a Vetter Model B reel-to-reel instrumentation tape recorder. Electric organ discharges recorded in this manner were converted to digital signals, and the power spectra of the signals were analyzed using a Zenith 286 microcomputer and ASYSTANT PLUS (Keithley Metrabyte) analytical software. Aliased frequencies represented in the generated power spectra were removed by software filtering.

Electric organ discharges (EODs) were elicited in skates by several different techniques. The methods employed included stimulation of the thoracic spinal cord either by insertion of a concentric bipolar stimulating electrode or *via* two monopolar stimulating electrodes located several centimeters distant from each other along the longitudinal axis of the cord. Alternatively, monopolar stimulating electrodes were placed at the rostral and caudal pores of the electric organ. Finally, EODs could be elicited through stimulation of the medullary electric organ command nucleus (EOCN) by means of an implanted concentric bi-

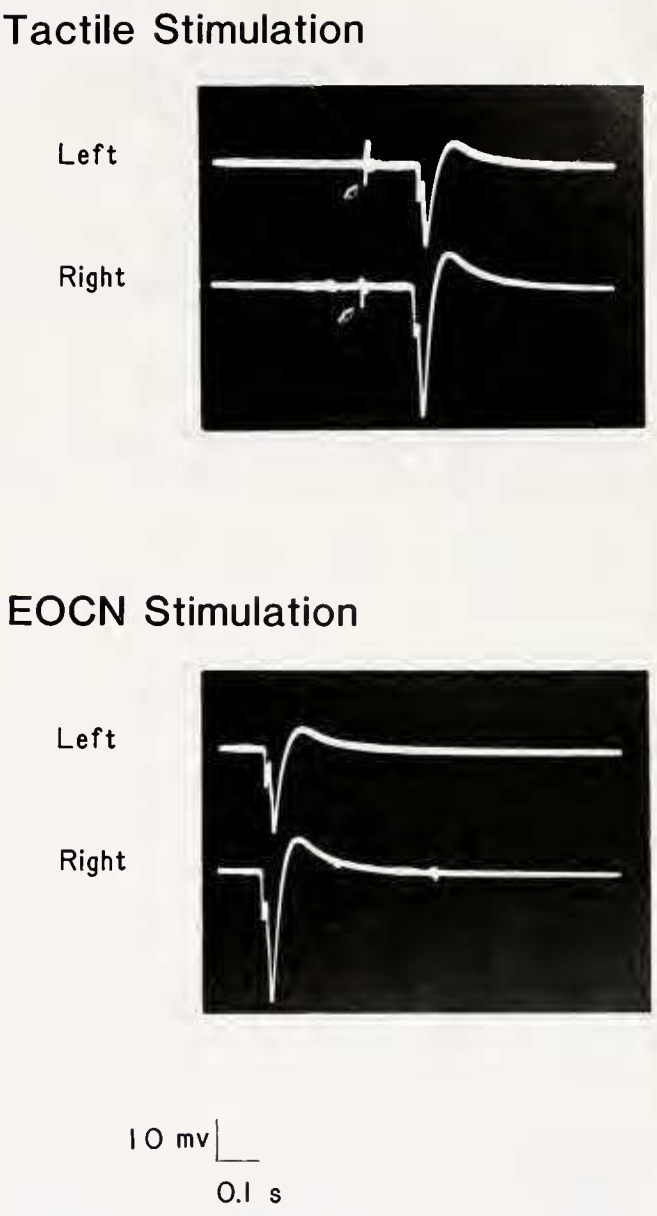

Figure 2. Electric organ discharges (EODs) measured simultaneously in right and left electric organs. Upper photo: EODs elicited by tactile stimulation (gentle tap of dorsal surface). Arrows indicate electromyograms of spinal reflexive withdrawal of pelvic fins following delivery of tactile stimulus. Lower photo: EODs recorded in the same animal by electric pulse train stimulation of the medullary electric organ command nucleus (EOCN). Stimulus delivery was 10 ms following initiation of the oscilloscope sweep.

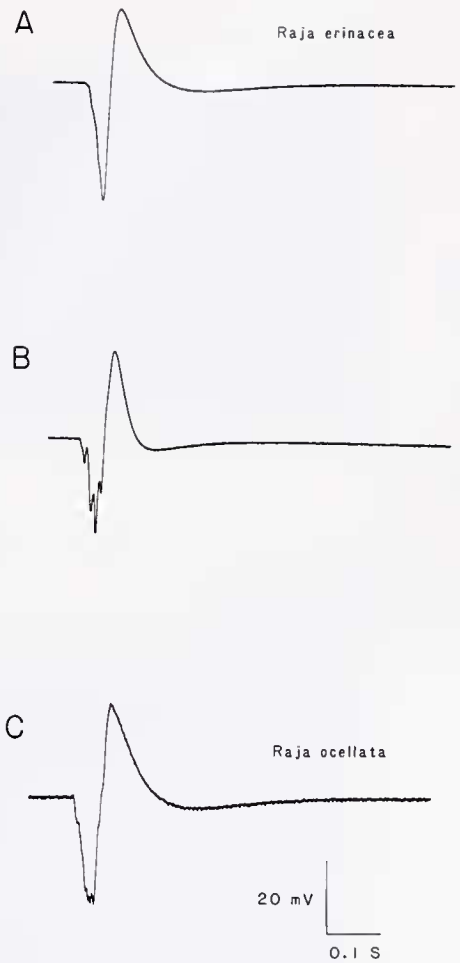

Figure 3. Electric organ discharges recorded from *Raja erinacea* (A, B) and *Raja ocellata* (C). The traces in (B) and (C) exhibit the multiple small peaks seen in many EODs evoked by both tactile and command nucleus stimulation.

polar electrode. In those cases in which the latter method of stimulation was employed, the trigeminal nerves were bilaterally transected to reduce movement caused by volume conduction of electrical current from the stimulating electrode to the nuclei of the branchiomeric motor column. Stimuli consisted of single DC step pulses or, more commonly, brief trains of pulses (3–5 pulses/train). The duration of the stimulus pulses was 0.5 ms, pulse amplitude 3–12 V; the pulse rate of the trains varied from 50–400 Hz, depending upon location; and the train duration was 40–70 ms.

Results

Electric organ discharges

Discharges of the electric organ (EODs) in the skate could be elicited by stimulation of the spinal cord, the electric organ, or the electric organ command nucleus; however, only the last method was found to be consistently reliable. Stimulation of the spinal cord with either a single concentric bipolar electrode or two monopolar electrodes placed at locations several centimeters distant from each other along the longitudinal axis of the cord was only occasionally successful in eliciting repeatable EODs, despite numerous repositionings of the stimulating electrode or electrode pairs. Neither single pulse stimuli nor trains of pulses up to 400 Hz elicited consistent EODs, despite the fact that they repeatedly and consistently elicited large motor neuron responses, as indicated by movement and electromyograms (EMGs) recorded *via* the subcutaneous electrodes planted in the tail. Similarly, concentric bipolar or paired monopolar electrodes implanted in the electric organ itself were only occasionally successful in eliciting EODs, presumably by means of the excitation of the presynaptic elements of the neural–electric organ junction.

Stimulation of the electric organ command nucleus (EOCN) was the most reliable method for eliciting consistent and repeatable EODs. Brief (50 ms) trains of 0.5-ms pulses delivered at 40–75 Hz and 3–5 V intensity were found to be sufficient stimuli. These were much lower frequencies and intensities (up to 15V and 500 Hz) than were required to occasionally elicit EODs by spinal cord or electric organ stimulation. The threshold of the EOD response to stimulation of the EOCN was dependent upon the location of the stimulating electrode; optimum results were found within an approximately 3-mm length of the basal medulla at the level of entry of the facial (VII) nerve.

The electric organ discharges recorded following stimulation of the EOCN were strongly similar in amplitude, duration, and waveform to those elicited by tactile stimulation (gently brushing or tapping the dorsal surface of the animal) and to the occasionally recorded spontaneous EODs generated by the animal (Fig. 2). Discharges in all cases were bilateral (both organs discharged) and synchronous, although occasionally an asymmetry in amplitude of the discharge was observed between the right and left electric organs. Such asymmetries occurred following both EOCN stimulation and tactile stimuli and persisted in multiple trials.

When measured across the rostrocaudal length of the electric organ by implanted subcutaneous electrodes, the EOD of both *R. erinacea* and *R. ocellata* consists primarily of a large peak that is negative with respect to the rostral pole of the organ, followed in most cases by a shallower and longer lasting positive component (Fig. 3). The mean amplitude of the negative peak for all waves recorded was −27.9 mV (SEM 2.58, $n = 35$), and the mean duration of the negative peak was 53.5 ms (SEM 2.16). The mean amplitude of the positive component of the EOD waveform was +11.12 mV (SEM 2.16, $n = 33$) and the mean duration was 192.35 ms (13.6 SEM). In both spontaneous EODs and those elicited by tactile or electrical stimulation,

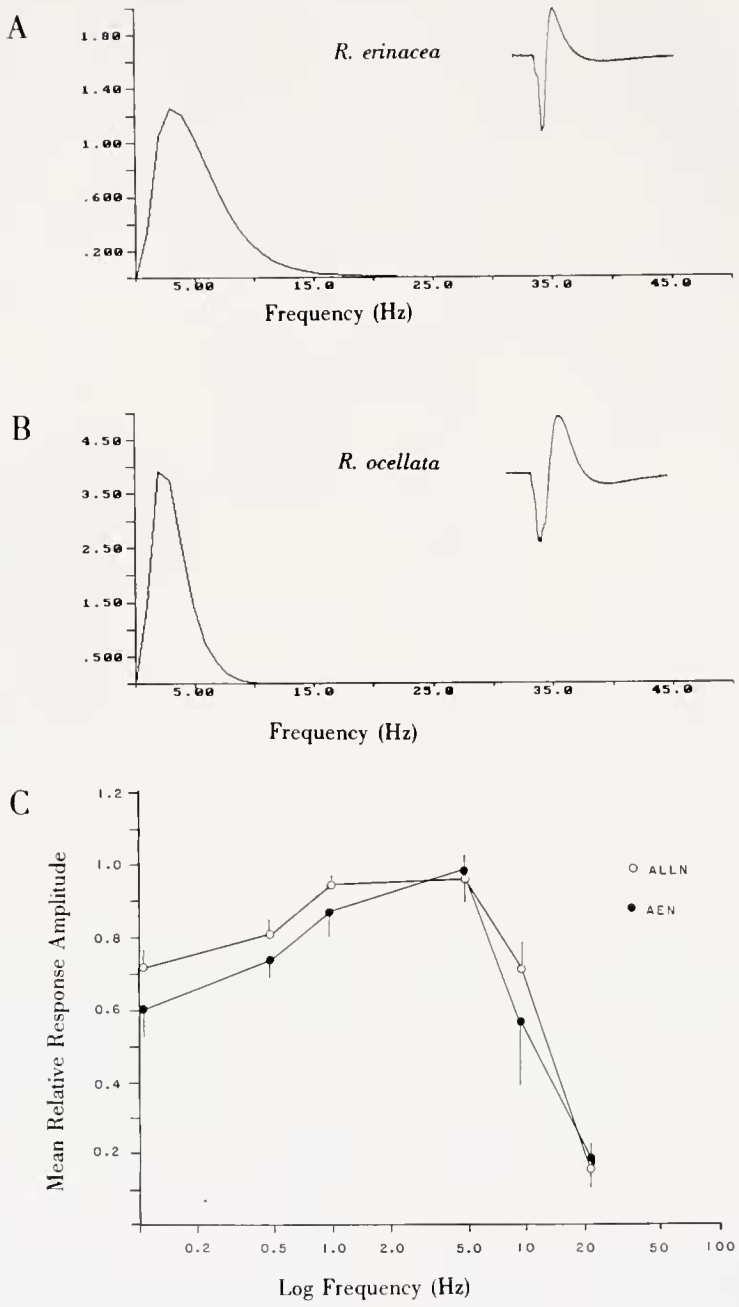

Figure 4. Power spectra of electric organ discharges recorded from *Raja erinacea* (A) and *Raja ocellata* (B) compared with frequency response curves generated for anterior lateral line nerve (ALLN) electrosensory afferent fibers and medullary ascending electrosensory neurons (AENs) (C). Bottom graph modified from New (1990).

there were frequently one or several small, sharp, positive peaks superimposed upon the slower negative wave (Fig. 3B). However, the shape of the waveform appeared to be very consistent for a specific individual, showing only minor variations, if any, between spontaneous and elicited EODs. The mean latency of the EOD of *R. erinacea* following EOCN stimulation was 89.4 ms (SEM 2.7, n = 26). The latency of EODs elicited by tactile stimulation was considerably longer than the responses of muscles that appeared to be involved in spinal reflexes, such as the pelvic fin withdrawal reflex. Figure 2 illustrates an EOD elicited by lightly tapping the animal's dorsal surface. The EMG recorded as the animal withdrew its pelvic fins can be observed at a considerably shorter latency (90 ms)

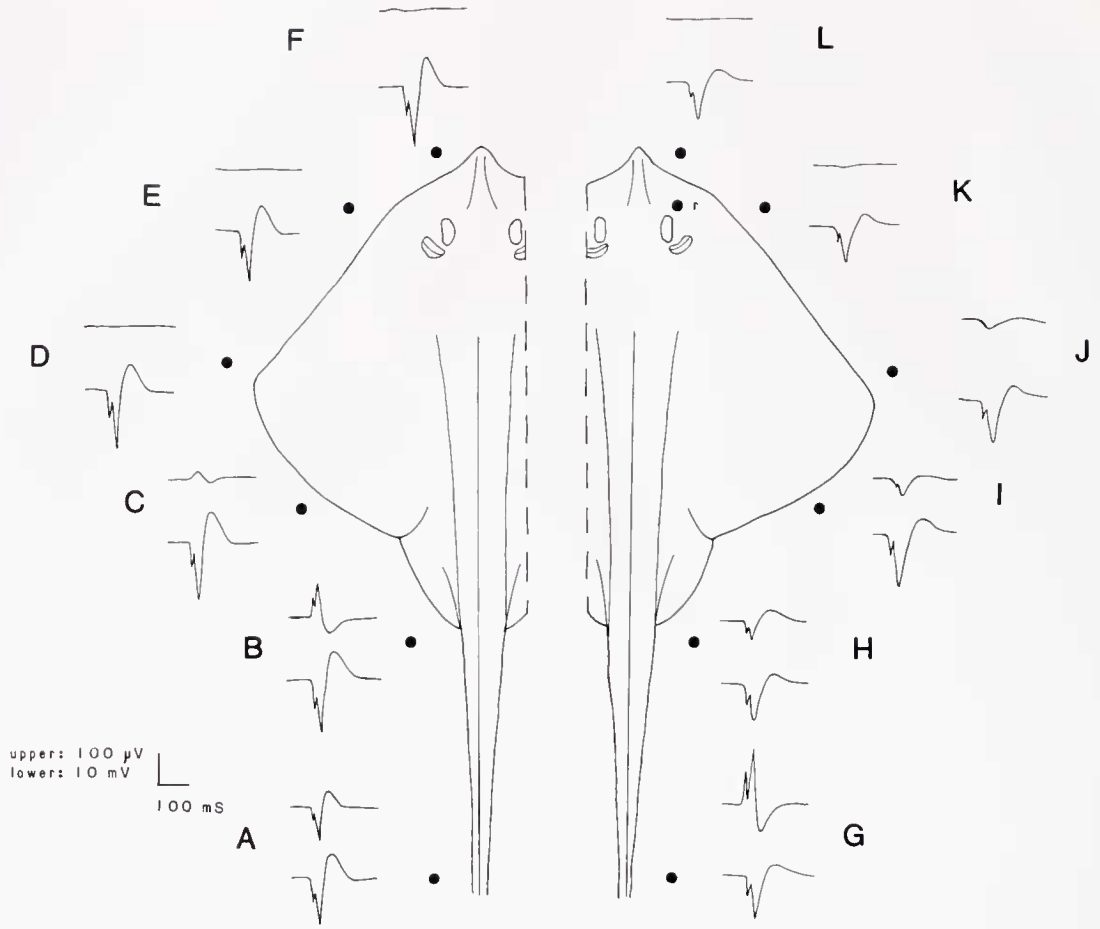

Figure 5. Field strength of the electric organ discharge elicited by EOCN stimulation measured around the body of the skate. Each pair of traces represents the field measured locally at the point indicated by the dot associated with each letter (top trace) and the EOD measured subcutaneously along the length of the electric organ (bottom trace). In traces A–F, the recording electrode was placed at the indicated locations and the reference electrode at a distant location. In traces G–L, the recording electrode was implanted in the ampullary tissues at the location marked "r" and the reference electrode at the positions indicated around the perimeter of the animal. In the latter examples, the voltage measured approximated the voltage drop along the length of the ampullary electroreceptors so oriented.

than the elicited EOD (235 ms), suggesting that somatosensory information must first ascend, presumably at least to medullary levels, rather than eliciting an EOD *via* a direct spinal reflex pathway to the electric organ motor neurons in the spinal cord.

The EODs of mature male and female specimens of *R. erinacea* and *R. ocellata* were recorded on tape and digitized for frequency composition analysis. The power spectra obtained from EODs of all specimens indicate that the majority of the power in the EOD signal is in a single range of frequencies between less than 1.0 Hz and 10–15 Hz, with the peak of the power spectra between 1.8 and 3.0 Hz (Fig. 4). Although the number of *R. ocellata* used in this study was too small for statistical analysis ($n = 2$), the mean duration and amplitude of the EOD of *R. ocellata* were within the range of those observed for *R. erinacea* (mean duration, negative peak = 50 ms, mean amplitude = −35 mV), and there appeared to be little difference in either the EOD waveform or power spectra between the two species.

The spread of the EOD around and through the skate was measured with external and internally implanted electrodes (Fig. 5). The externally measured field created by the EOD reverses its polarity across the length of the electric organ and diminishes sharply with increasing distance around the surface of the animal from the rostral terminus of the organ, diminishing to less than millivolt intensity at the rostral end of the skate's body (Fig. 5A–F). The amplitude of the externally measured EOD at the rostral terminus of the organ, at a lateral distance of 1 cm from the tail, was 180 μV peak to peak. At a distance of 4.6 cm from the rostral tip of the electric organ along

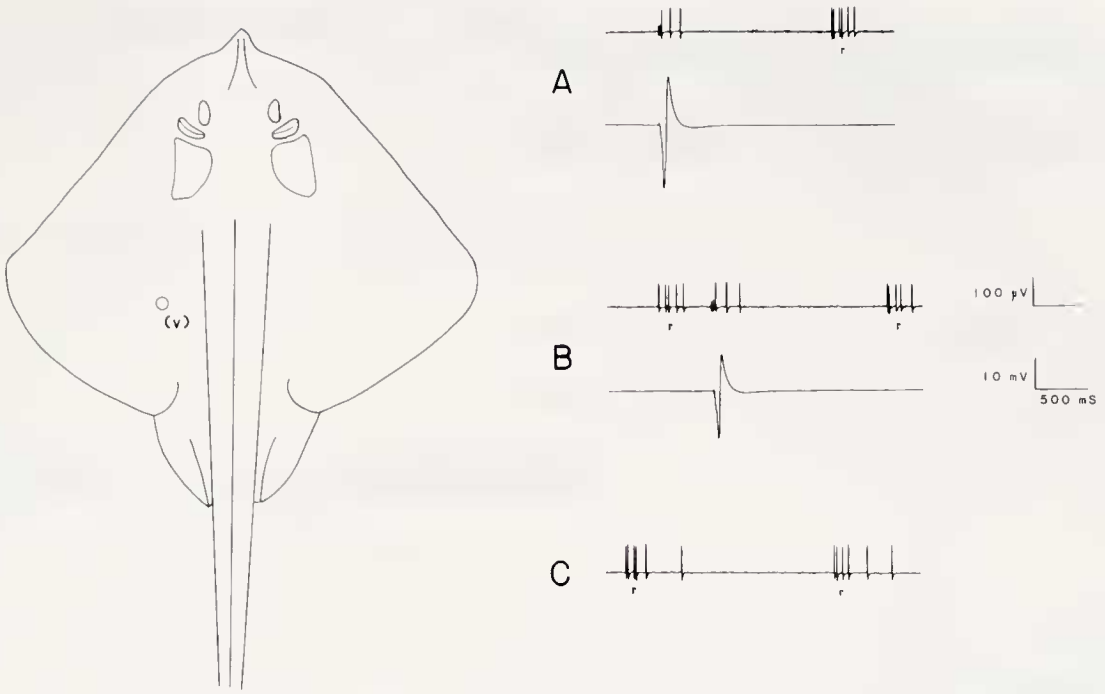

Figure 6. Responses of a single electrosensory ALLN afferent fiber to an elicited EOD. The location of the epidermal pore of the ampullary electroreceptor innervated by this particular afferent is shown at the location marked "(v)" (the pore was located upon the ventral surface of the skate). The small series of waves immediately preceding the EOD-invoked reafferent burst is a stimulus artifact of the train pulse delivered to the EOCN. The bursts of activity indicated with the letter "r" indicate reafferent activity caused by the animal's ventilation.

the pectoral disk, the amplitude had decreased to 50 μV, and at 8.7 cm (at the widest diameter of the pectoral disk) the peak-to-peak amplitude had decreased to less than 5 μV. A recording electrode was subsequently implanted inside the animal in the region of the superficial ophthalmic ampullary cluster. The EOD thus measured as the difference across the skin of the skate (*i.e.*, across the length of the receptors) was similar to the externally measured field in amplitude and decrease in amplitude with distance from the electric organ, but the polarity of the recorded discharge was reversed (Fig. 5G–L). The latter results indicate that the electric organ discharge does not generate a significantly strong internal field contrary to that generated during the animal's ventilatory activity.

Responses of electrosensory ALLN afferent fibers

A total of 32 individual ALLN fibers with identified ampullae were recorded in this study. The responses of electrosensory ALLN fibers innervating different ampullary electroreceptors displayed considerable variability, depending upon the location of the ampullary pore. Ampullary organs opening through caudally directed canals to pores located upon the caudal surface of the pectoral fin showed the greatest responses (Figs. 6, 7). The maximal instantaneous frequency of the action potential response recorded from these afferents was approximately 12 spikes/second. Ampullary organs with more rostrally located pores showed responses with a much lower signal-to-noise ratio (Fig. 8). The responses of the most rostrally located and directed organs (the majority of ampullary electroreceptors in the skate) showed very weak responses to electric organ discharges, or no detectable response above the normal resting activity of the ALLN fiber.

Additionally, the temporal pattern of the measurable responses of identified electrosensory ALLN afferents varied depending upon the location and orientation of the ampullary organ canal and epidermal pore. Generally, fibers innervating the most caudally oriented and located ampullary organs responded with a brief burst of action potentials during the positive-going phase of the EOD (Fig. 7). The response pattern for units innervating more-rostral ampullary organs is less clear owing to the low signal-to-noise ratio of the response and the ongoing activity of the fiber. However, several of these neurons demonstrated a response that was out of phase with the more caudal units, *i.e.*, an inhibition of activity associated with the EOD. In no case was the response of electrosensory ALLN fibers to discharge of the electric organ as robust as the response of these same units to the potentials gen-

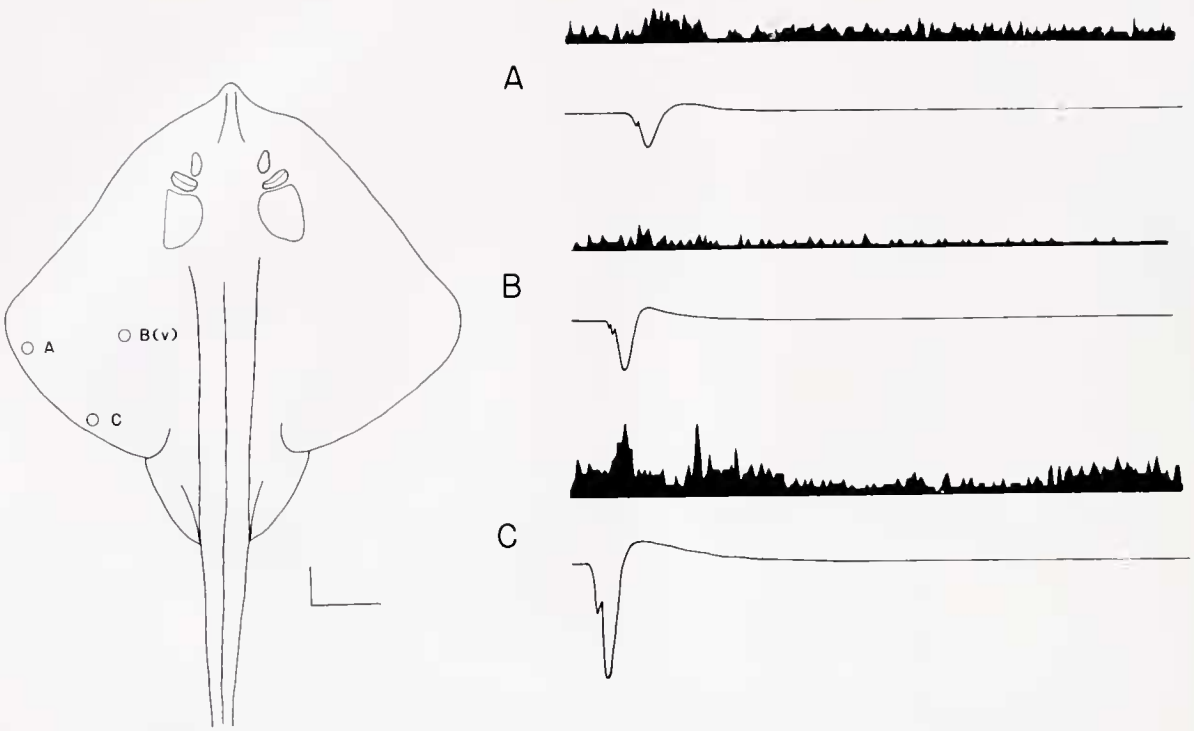

Figure 7. Peristimulus time histograms (PSTHs) of EOD-invoked reafference in three ALLN electrosensory afferent fibers innervating caudally oriented ampullary receptors. The ampullary pores of the receptors are indicated by the open circles; "v" indicates a pore on the ventral surface of the pectoral disk. The horizontal time scale equals 100 ms; the vertical scale indicates instantaneous frequencies of 17.5 spikes/s (A, B) and 7.5 spikes/s (C).

erated by the animal's ventilatory activity (Fig. 6). The maximal spike rate of the responses to respiratory potentials in ALLN fibers approached 91 spikes/second.

Discussion

Reafference produced as a result of motor activity plays an important role in the activity and organization of nervous systems. Ventilatory activity in skates produces a strong reafference, driving the electrosensory ALLN afferents through approximately 63% of their dynamic range (New and Bodznick, 1990). Reafference of this intensity may well interfere with the animal's ability to detect externally generated fields of interest to the animal. In the central nervous system of elasmobranchs, electrosensory reafference produced by ventilatory activity (gilling) is reduced through a common-mode rejection mechanism based upon commissural and unilateral local circuit neurons in the first-order medullary nucleus (Montgomery, 1984b; New and Bodznick, 1990; Bodznick and Montgomery, 1992). Unlike the common-mode reafference produced by ventilation, in which all fibers are excited and inhibited in phase together, the response of electrosensory ALLN afferents to the EOD is highly variable among fibers innervating different ampullary receptors, both in the intensity of the response and in the temporal pattern. Invariably, the reafference produced by discharge of the electric organ is much weaker than that generated by ventilation; the maximal response of the most caudally directed ampullary organs drives the fibers through less than 10% of their dynamic range. Indeed, for most of the electroreceptors, which are located in the rostral third of the pectoral disk, the responses of innervating ALLN fibers to the EOD are difficult to detect against the ongoing activity of the nerve.

The variability in intensity and temporal pattern of ALLN fiber responses indicates that the reafference produced by electric organ discharge is fundamentally different from that produced when the animal ventilates. Respiratory potentials are produced by the modulation of DC potentials generated by the different electrical properties of various body surfaces in contact with the surrounding seawater, particularly ion-exchange surfaces. Potentials thus produced are probably modulated by changes in the resistance of the current pathway caused by the opening and closing of mouth and gill slits during ventilation (Kalmijn, 1988; Bodznick *et al.*, 1992). These potentials modulate the activity of ampullary organs by changing the voltage at the *internal* surface of the apical

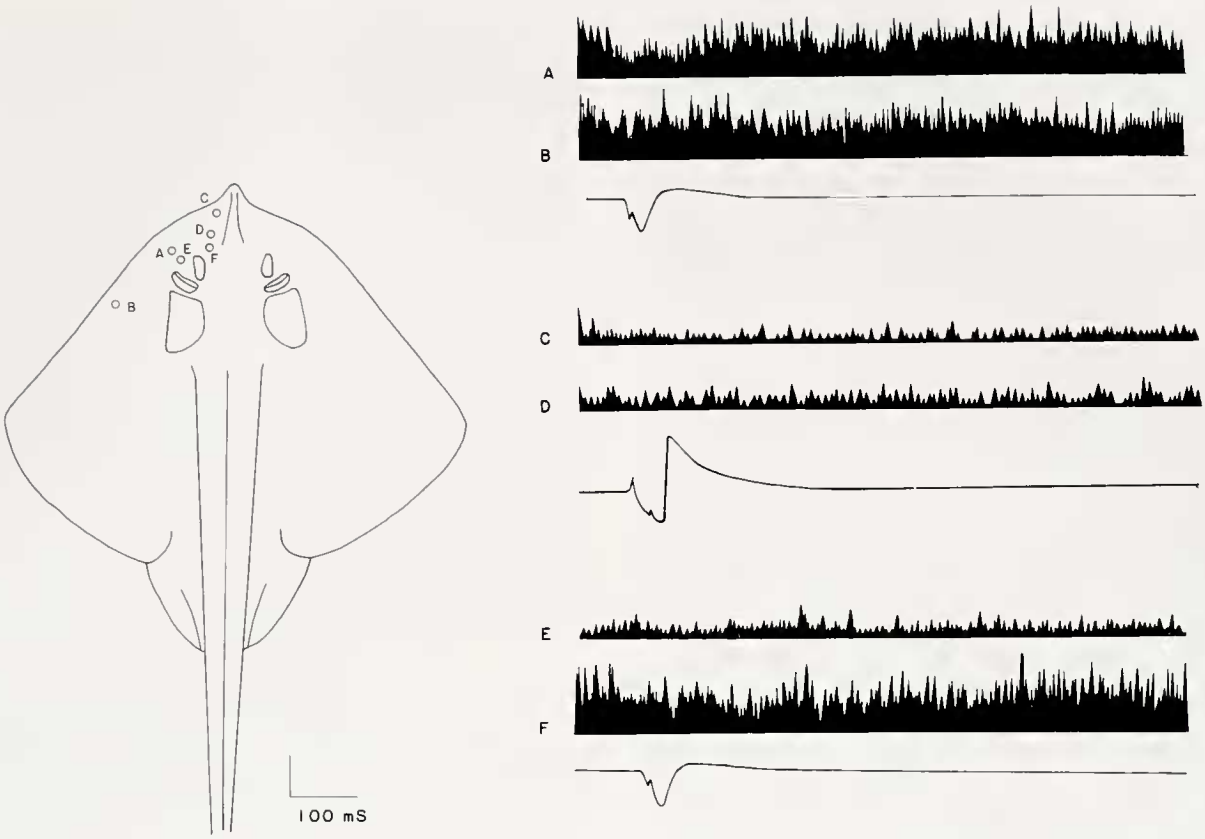

Figure 8. Peristimulus time histograms (PSTHs) of EOD-invoked reafference in ALLN electrosensory afferent fibers innervating rostrally located ampullary receptors. The ampullary pores of the receptors are indicated by the open circles; the pores were located on either the dorsal or ventral surface of the pectoral disk. The horizontal time scale equals 100 ms; the vertical scale indicates instantaneous frequencies of 20.0 spikes/s (A, B, F) and 17.1 spikes/s (C, D, E).

membrane of the receptor cells by means of current flow through a low-resistance pathway in the visceral tissues of the skate. All receptor cells are thus modulated in phase together from the inside, regardless of the orientation of the receptor's canal or the location of the ampullary pore on the surface of the body. Such is clearly not the case for reafference produced by the EOD.

The variability of the responses of ALLN fibers to EOD reafference suggests that the EOD spreads over the outside and inside of the animal and modulates the activity of ampullary electroreceptors differentially depending upon receptor location and orientation with respect to the generated external electric field. The experiments measuring the shape and intensity of the field generated by the EOD support this view. When measured with respect to a distant (approximately 25 cm) reference electrode, the strength of the field drops off rapidly with increasing distance from the electric organ, possibly dropping off to below the detectable range of elasmobranch electroreceptors within several body lengths. When the recording electrode is implanted within the tissues of the ampullary cluster, and the voltage drop along the length of the receptor is measured by moving the reference electrode to various locations around the animal's epidermis, a similar decrease in voltage with distance from the electric organ was observed, although the polarity of the recorded EOD was reversed. These results indicate that the EOD does not modulate the activity of electroreceptors internally, as do the potentials produced during ventilation, but rather through an external and internal spread of the EOD over the surface and through the tissues of the animal. It is possible that the electric organs are insulated from the low-resistance tissues of the viscera by the surrounding layers of muscle and connective tissue sheathing of the EOD in the skate's tail.

Although it is difficult to discern the responses of many individual electrosensory ALLN fibers to the EOD against the ongoing activity of the fiber, the peaks of the EOD power spectrum fall within the bandwidth to which ampullary electroreceptors are most sensitive (1–5 Hz) and suggest that the EOD should be an effective stimulus at short ranges. Although the signal-to-noise ratio of primary

afferent fiber responses to EOD appears to be low when compared to that generated by ventilation, these fibers have a high, steady rate of resting activity and, in these experiments, are being strongly modulated by the fish's ventilatory potentials. Physiological studies in *Raja* and in other elasmobranchs have shown that the ascending electrosensory neurons (AENs) in the first-order medullary nucleus have a substantially higher signal-to-noise ratio for weak external fields. This higher ratio is the result of a low resting activity and the presence of central mechanisms for the suppression of ventilatory reafferent interference (Montgomery, 1984b; New and Bodznick, 1990; Bodznick and Montgomery, 1992). Furthermore, it has been demonstrated in elasmobranchs and other electroreceptive fish that behavioral thresholds for electric field detection are substantially lower than those measured physiologically, particularly at the level of the hindbrain. Thus, the responses of central neurons to electric organ discharges, produced as reafference or by another skate, may be substantially greater than those recorded in the ALLN fibers. Indeed the ability of male stingrays to detect the very much smaller respiratory potentials of female conspecifics at considerable distances (up to 1 m) (T. Tricas, Florida Inst. Tech., in prep., cited with permission) argues that the electric organ discharges of skates may be effective signals at considerable distances.

Although several studies have examined the production in different circumstances of electric organ discharges in skates, no behavioral studies have conclusively demonstrated a behavioral role for the EOD. Mikhailenko (1971) presented limited evidence that EODs may play a role in social communication by demonstrating seasonal variations in EOD structure. From these data he suggested a relationship between EOD changes and seasonal reproductive cycles. Mortenson and Whitaker (1973) reported that the frequency of electric organ discharge was greater from pairs of skates swimming together than for skates held singly; these authors also noted an increased frequency of discharge during the dark portion of the light: dark cycle. Bratton and Ayers (1987) observed that skates produced EODs in response to presentation of tactile or electric field stimuli and also when physically encountering other skates. All of these studies strongly suggest a social communication function for the EOD, but the lack of any overt behavior typically associated with discharge of the electric organ makes it difficult to define a contextual behavioral role for these discharges. The temporal structure and power spectra of the EODs of male and female *Raja erinacea* and *Raja ocellata* recorded in this study were very similar, which would seem to preclude gender or species identification by EOD identification. The results of this study differ somewhat from those of Mortenson and Whitaker (1973) and Bratton and Ayers (1987), in that the time course of EODs recorded from the two specimens of *R. ocellata* was very similar to that recorded from *R. erinacea*. Such a difference may be related to the method by which EODs were elicited in this study (electrical stimulation of the EOCN in a decerebrate animal), although EODs evoked by tactile stimulation were invariably very close to those elicited by EOCN stimulation. Alternatively, seasonal variation in the structure of the EODs of *R. ocellata* or the low number of specimens used in this study may account for the observed discrepancy, because variability in the EOD of *R. ocellata* has been observed in other studies (Bratton *et al.*, 1993). Studies in other fishes with weak electric organs have demonstrated that both the pattern of the EOD and the sensitivity of the electrosensory system can be modified by increased androgen secretion associated with sexual maturity (Meyer and Zakon, 1982; Meyer, 1983; Zakon and Meyer, 1983; Bass and Hopkins, 1984). However, changes in the EOD structure and receptor sensitivity have not been tested for in the reproductive cycles of skates.

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